

Assessment of anti-SARS-CoV-2 antibodies post-Coronavac Vaccine in the Amazon region of Brazil

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Abstract

Background. The race to develop a protective vaccine for SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) has raised questions regarding the safety of immunizers, the efficacy in the production of neutralizing antibodies, the longevity of the immune response, especially when related to variables such as sex and age.

Objective. The present study evaluated the frequency of seroreactivity for anti-SARS-CoV-2 (S1 and S2) total antibodies and anti-SARS-CoV-2 (RBD - S1) neutralizing antibodies in individuals vaccinated with the immunizing agent Coronavac (Sinovac).

Method. The study was cross-sectional and involved a total of 358 individuals divided into two groups. Group 1 consisted of 205 volunteers who were tested for anti-SARS-CoV-2 total antibodies (S1 and S2) and group 2 of 153 individuals tested for the presence of anti-SARS-CoV-2 neutralizing antibodies (RBD - S1).

Results. Seroreactivity was above 70% in both groups, with approximately 20% of individuals showing no reactivity. The frequency of anti-SARS-CoV-2 total antibodies (S1 and S2) showed a significantly different distribution between sexes, but not according to age. The frequency of anti-SARS-CoV-2 (RBD - S1) neutralizing antibodies was 100% in the age group from 20 to 40 years, reducing significantly with advancing age to 88.9% (41 to 60 years), 78.7% (61 to 80 years) and 58.3% (>80 years).

Conclusion. Our results demonstrate a high prevalence of anti-SARS-CoV-2 total antibodies (S1 and S2) and anti-SARS-CoV-2 (RBD - S1) neutralizing antibodies in individuals who received both doses of Coronavac vaccine, suggesting a lower efficiency of the humoral immune response among the elderly over 60 years of age, which could be associated with the senescence of the immune system.

Background

The SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) pandemic that emerged in Wuhan, China in November 2019 (WHO, 2020) brought a series of concerns, questions and lessons (Chen et al. 2021). At the same time, it was possible to experience a race, never before seen in the history of world science, for the development of a vaccine capable of eliciting neutralizing antibodies against SARS-CoV-2, reinfections by different variants of the virus (Rubin, 2021), and confer protection against severe cases of COVID-19 (Coronavirus Diseases 2019) (Corey et al. 2020). At this point, new anti-SARS-CoV-2 vaccine development platforms were implemented, using inactivated virus, non-replicating viral vector, subunit, Viral like particle, DNA and mRNA (Park and Lee, 2021), which generated hundreds of records of preclinical and phase II, III, and IV clinical studies (WHO, 2021). Along with the proposals for new technologies for the creation of anti-SARS-CoV-2 vaccines, there were also doubts and concerns (Fedele et al. 2021), especially regarding the safety and efficacy of the new platforms which, until then, were not used in humans (Blumental and Debré, 2021).

Parallel to advances in understanding the immunological mechanisms present in SARS-CoV-2 infection and in the modulation of COVID-19, phase II and III studies showed satisfactory and promising efficacy and safety results for different anti-SARS-CoV-2 vaccine platforms (Park and Lee, 2021), which allowed that, at the moment, 14 vaccines are in use among different countries.

In Brazil, ANVISA (National Health Surveillance Agency), a government agency responsible for regulating pharmacological and immunobiological inputs, approved in January 2021, the definitive registration of the Pfizer

and AstraZeneca vaccines. Coronavac (Sinovac) and Janssen-CILAG vaccines are approved only for emergency use (ANVISA, 2021).

The main question and the reason for different opinions and discussions is about the effectiveness of mass vaccination campaigns, especially in regard to the efficiency of immunizers in generating protective immunity and immunological memory. This discussion highlights the relationship of variables such as sex and age, since the immune response can have different dynamics when considering sexual dimorphism (Ulhaq et al., 2020) and the physiological senescence of the immune system (Ciarambino et al., 2021). In this context, serological studies have been carried out to assess population efficiency in generating post-vaccination neutralizing antibodies (Edara et al., 2021).

The present study assessed the frequency of anti-SARS-CoV-2 total antibodies specific for the S1 and S2 portions of the viral spike protein, as well as anti-SARS-CoV-2 (RBD - S1) neutralizing antibodies in two independent groups of individuals who sought care at the Amaral Costa Medicina Diagnóstica laboratory, after having received the second dose of Coronavac vaccine.

Methods

Studied samples

This was a cross-sectional study in which blood samples were collected from March to April 2021 and included 358 individuals (Table 1), of both sexes (138 males and 220 females), aged between 21 and 96 years (average 66.6 years) who voluntarily sought the Amaral Costa Medicina Diagnóstica laboratory in the city of Belem, capital of the State of Para (Northern Brazil) 30 days after the second dose of Coronavac vaccine (Sinovac Research and Development Co. Ltd/Butantan). Of the total number of individuals analyzed, 205 performed anti-SARS-CoV-2 total antibody test (S1 and S2) and 153 were tested for anti-SARS-CoV-2 neutralizing antibody (RBD - S1).

Ethical aspects

The study was approved by the Ethics and Research in Human Beings Committee of the Health Sciences Institute of the Federal University of Pará (CAAE: 31800720.1.0000.0018) in compliance with the guidelines and regulatory standards for research involving human beings, in accordance with the Declarations of Helsinki.

Antibody analysis

Whole blood samples (5mL) were collected in vacuum tubes without anticoagulant. Serum samples were separated by centrifugation. Investigation of anti-SARS-CoV-2 total antibodies (S1 and S2) was performed by qualitative microparticle chemiluminescent immunoassay (CMIA) on the LIAISON® XL Analyzer automated platform (DiaSorin, Saluggia, VC, Italy), following the manufacturer's recommendations. The reference ranges were non-reactive (<12 AU/ml), indeterminate ($12.0^3 \times <15.0$ UA/ml) and reagent (>15.0 AU/ml).

Anti-SARS-CoV-2 (RBD - S1) neutralizing antibodies was performed using the competitive enzyme immunoassay GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript, FDA, EUA), following the manufacturer's protocol. The approach also known as SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit, is a faster, easier, more scalable and automatable alternative to the traditional neutralizing antibody tests, such as

virus neutralization test (VNT), pseudo-virus neutralization test (pVNT) and plaque reduction neutralization test (PRNT). The reference ranges used were non-reactive (<20%), indeterminate (20% x £29%) and reagent (>30%).

Statistical analysis

Information on sex, age and antibody status were tabulated in Excel software. The calculation of antibody frequencies was performed by direct counting and the significance of comparisons between groups was assessed by the chi-square and G tests, using the Bioestat version 5.3 program. Differences were considered statistically significant when the p value was <0.05.

Results

The presence of anti-SARS-CoV-2 total antibodies and anti-SARS-CoV-2 neutralizing antibodies showed that 270 (75.4%) presented positive results, 70 (19.6%) were non-reactive and 18 (5.0%) indeterminate (Table 1).

Table 1

Frequency of anti-SARS-CoV-2 total antibodies (S1 and S2) and anti-SARS-CoV-2 neutralizing antibodies (RBD - S1) following two doses of Coronavac in the investigated groups in Belem, Para.

Sample	Vaccine	Test	N	Male	Female	Age	Reagent (%)	Indeterminate (%)	Negative (%)
Group 1	Coronavac	Total Antibodies	205	77	128	65.5	159 (77.6%)	03 (1.5%)	43 (20.9%)
Group 2	Coronavac	Neutralizing antibodies	153	61	93	65.4	153 (72.6%)	15 (9.8%)	27 (17.6%)
Groups 1 and 2	Coronavac	Total and Neutralizing antibodies	358	138	220	65.4	270 (75.4%)	18 (5.0%)	70 (19.6%)

Those who were tested for anti-SARS-CoV-2 total antibodies (205 individuals), 159 (77.6%) individuals had a reactive result, 43 (20.9%) were non-reactive and 3 (1.5%) indeterminate (Table 1). Those who were tested for anti-SARS-CoV-2 neutralizing antibodies (153 individuals), 111 (72.6%) presented a reactive result, 27 (17.6%) were non-reactive and 15 (9.8 %) indeterminate (Table 1).

The reactivity according to the results of both tests, showed a significantly higher value ($p=0.0022$) in females (85%) when compared to males (70.7%; Figure 1A). A similar profile was observed ($p=0.0041$) for the 205 individuals who underwent testing for anti-SARS-CoV-2 total antibodies (Figure 1B). The frequency of anti-SARS-CoV-2 neutralizing antibodies did not show significant differences between sexes (Figure 1C).

The reactivity profile according to the results of anti-SARS-CoV-2 total antibodies plus anti-SARS-CoV-2 neutralizing antibodies (Figure 1A) showed significant differences with age groups ($p=0.0084$). The highest frequency occurred in the age group of 20-40 years (94.1%) and then gradually decreased to 87.7% (41-60 years), 76.8% (61-80 years) and 68.4% (>80 years) as the age increased. These differences were not observed when measuring anti-SARS-CoV-2 total antibodies (Figure 1B); but followed the same pattern when measuring only anti-SARS-CoV-2 neutralizing

antibodies ($p=0.0218$; Figure 1C), in which individuals aged 21 to 40 years showed 100% reactivity and decreased gradually with advancing age to 88.9% (41 to 60 years), 78.7 % (61 to 80 years) and 58.3% (>80 years).

There were no significant differences detected between sexes and age group in regard to reference values for anti-SARS-CoV-2 total antibodies (UA/mL) and anti-SARS-CoV-2 neutralizing antibodies (% neutralization) (Figure 2 A and B).

Discussion

The prevalence of seroreactivity for anti-SARS-CoV-2 total antibodies and anti-SARS-CoV-2 neutralizing antibodies was evaluated in the present study in individuals vaccinated with two doses of Coronavac. The results were similar, regardless of the method of choice to assess humoral immunological response, including the frequency of persons who did not present antibodies. The seropositivity values are lower than those reported by the manufacturer of the immunizing agent during phase I and II randomized clinical trials in adults, young people and elderly people over 60 years and higher than the value of vaccine efficacy reported in health care professionals in direct contact with patients with COVID-19 (Instituto Butantan, 2021). A limitation of the present study was the lack of information on the occurrence of previous infection in vaccinated individuals, a variable which might interfere in the assessment of post-vaccination seroconversion.

A recent meta-analysis study showed that the Coronavac vaccine would be efficient in generating neutralizing antibodies (Rogliani et al., 2021), which together with the present results, particularly those obtained for anti-SARS-CoV-2 neutralizing antibodies, are encouraging, as they suggest that mass vaccination of the population with Coronavac can generate collective protection (Omer et al., 2020), considering the percentage of reactivity observed in the present study.

Anti-SARS-CoV-2 vaccines has generated different opinions and discussions about the efficacy and efficiency of these immunizers in relation to the potential for generating protective immunity and persistence of immunological memory (Blumental and Debré, 2021; Widge et al., 2021), especially with regard to variables such as sex and age. Considering that the immune response may have distinct dynamics supported by factors related to sexual dimorphism (Ulhaq et al., 2020; Jin et al. 2021) and physiological senescence of the immune system (Ciarambino et al., 2021), the frequency of anti-SARS-CoV-2 total antibodies observed in the present study was significantly more frequent in females, which corroborates the literature describing females, when compared to males, as presenting increased inflammatory and humoral responses in response to COVID-19 (Ciarambino et al., 2021).

Furthermore, in regard to seroreactivity for anti-SARS-CoV-2 neutralizing antibodies, it was observed high prevalence among young-adults, and lowest frequency among the elderly, which may suggest a lower efficiency of the vaccine to stimulate humoral immune response among elderly individuals. It reinforces evidence that in elderly patients, there is a functional and progressive decline in the immune system (Ciarambino et al., 2021). It is important to emphasize that the absence of detection of a post-vaccination humoral immune response does not mean, however, the absence of immunity to SARS-CoV-2, since the serological methods used do not assess the presence of cellular immunity ($CD4^+$ and $CD8^+$ T lymphocytes), which may be present even in the absence of antibodies (Grifoni et al., 2020; Gallais et al., 2021).

Conclusion

The results presented herein demonstrate a similar pattern in the frequency of seroreactivity of anti-SARS-CoV-2 total antibodies and anti-SARS-CoV-2 neutralizing antibodies in individuals who received two doses of the Coronavac vaccine. There was a lower efficiency of humoral immune response among the elderly, which could be associated with the senescence of the immune system. It is important that this information is confirmed as it might be necessary to administer a third dose of the vaccine in this group, especially due to their greater vulnerability to the most severe clinical COVID-19.

Finally, considering the emergence of viral variants, the neutralizing antibody response after vaccination should be monitored. Our results support the performance of population-based serological studies aimed at better understanding the efficiency of vaccines approved for use in Brazil in terms of their ability to generate herd immunity against SARS-CoV-2.

Abbreviations

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus; COVID-19: coronavirus disease 2019; SARS: Severe acute respiratory syndrome; IgG: Immunoglobulin G; CMIA: Chemiluminescent microparticle immunoassay; ELISA: Enzyme-linked immunosorbent assays; RBD: Recepto binding domain; S1: spike 1; S2: spike 2.

Declarations

Ethics approval and consent to participate

This project was submitted to and approved by the Human Research Ethics Committee of the Institute of Health Sciences of the Federal University of Pará (CAAE: 31800720.1.0000.0018) in compliance with the guidelines and regulatory standards for research involving human beings. Individuals who agreed to participate in the study signed an informed consent form.

Consent for publication

Not applicable.

Availability of data and materials

Data are available upon request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ACRV and CDAB conceived the project. CDAB, IPCA, and GLV performed the laboratory analyses. CDAB, ESGA, and MAFQ performed the statistical analyses and wrote the draft of the article. ACRV, RI, CNCB and IMVCV reviewed the article.

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Figures

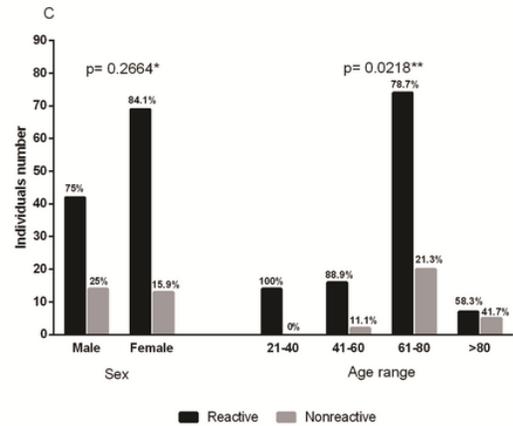
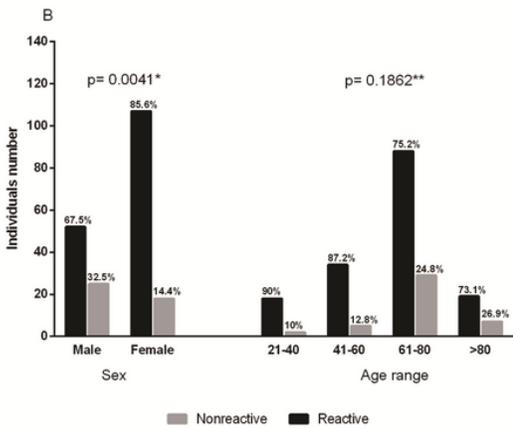
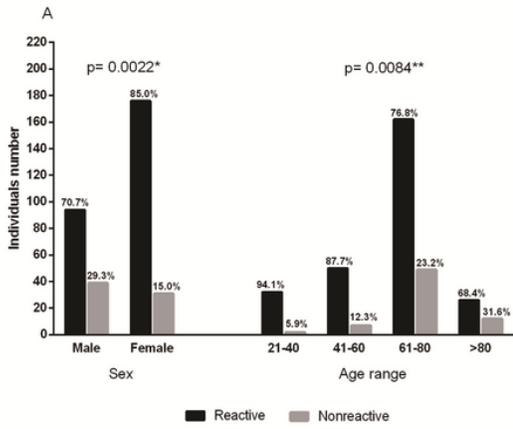


Figure 1

Frequencies of anti-SARS-CoV-2 antibodies according to sex and age group. (A) Pooled frequencies of anti-SARS-CoV-2 total antibodies (S1/S2) plus anti-SARS-CoV-2 IgG neutralizing antibodies (RBD - S1). (B) Frequencies of total anti-SARS-CoV-2 antibodies (S1/S2). (C) Frequencies of neutralizing IgG anti-SARS-CoV-2 (RBD - S1) antibodies. *Chi-square test; **Test G.

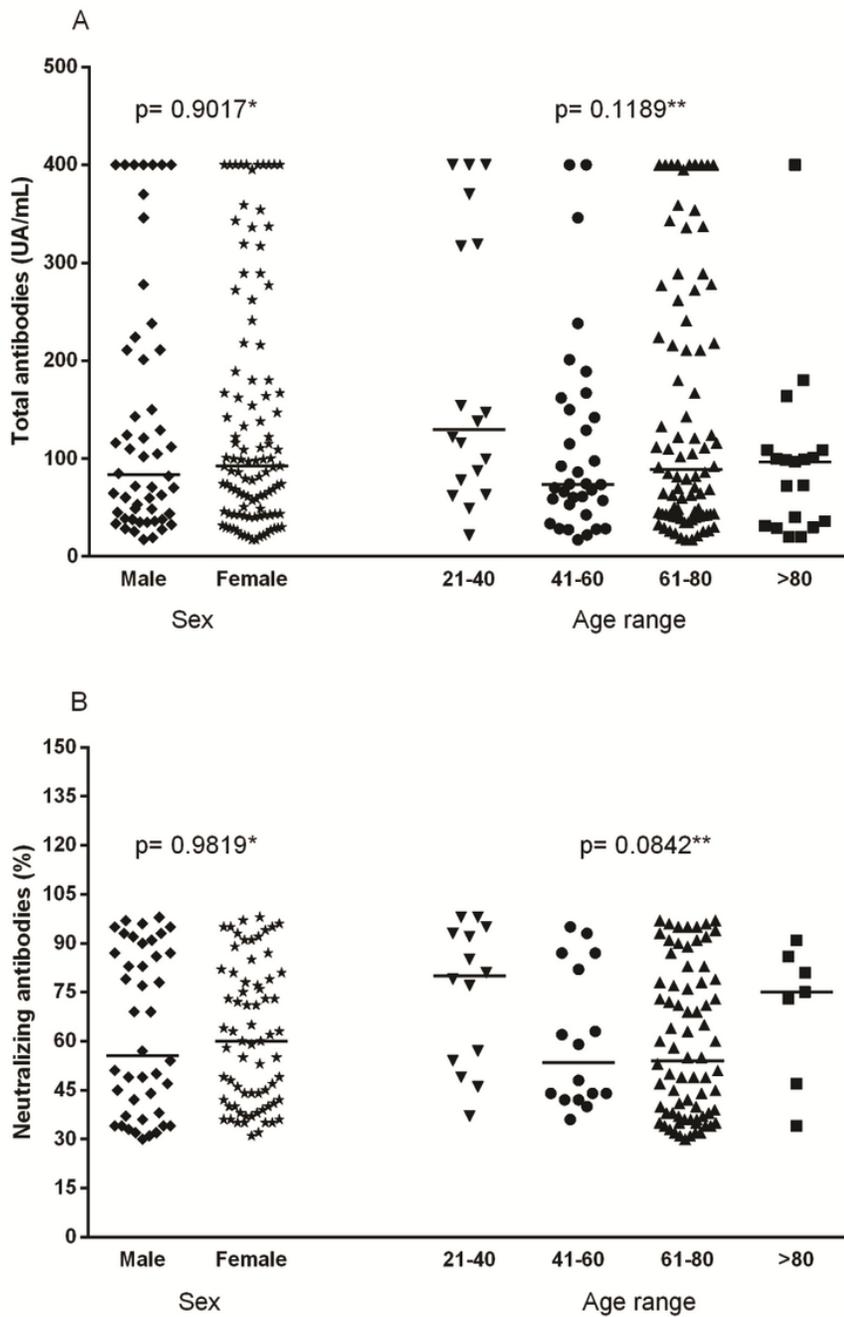


Figure 2

Comparison of reference values for antibodies between sexes and age groups. (A) Anti-SARS-CoV-2 total antibodies (S1/S2) (UA/ml) and (B) anti-SARS-CoV-2 IgG neutralizing antibodies (RBD - S1) (% neutralization). *Mann Whitney Test; **Kruskal-Wallis test.